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Statistical presentation of results of the complement fixation test in toxoplasmosis

Statystyczne ujęcie wyników odczynu wiązania dopełniacza
w toksoplazmozie

Toxoplasmosis is a widely distributed zoonosis which is transmitted to the man by the oral, inhalation or dermal routes. It runs in the man usually a mild course, symptomfree although sometimes appear forms with acute manifestations. In gynecology the role of toxoplasmosis is expressed by causing women affected by it not complete pregnancy, deliver prematurely children or stillborns with developmental abnormalities (Aresin, 1957).

Toxoplasmosis cannot be diagnosed on the sole basis of clinical symptoms. The clinical diagnosis must be confirmed by laboratory examinations. The introduction of uniform laboratory methods in all countries is a matter of great importance for the combating of this disease.

Using the intradermal test the greatest occurrence of invasions was found in Mexico 47,6% (Biagi), Sweden 40—46% (Gard, Hedgnist), Austria 46% (Thalhammer). Relatively lower results were recorded in England 17% (Fischer), Germany 11—26% (Keller, Vivell, Falck, Müller), Spain 27,6% (Sotller, Durall, Vilardell, Finas), Italy 16,7% (Tolentino et Razzi), USA 10—31% (Frenkel, Adams, Feldmann, Sabin), South Africa 31% (Schneider — cited after O. Jirovec). In Poland, in the Gdańsk and Białystok Palatinates the percentage of invasions amounts to 25 (Kozar), in the Lublin Palatinate 34 (the authors studies).

In the literature the authors citing various percentages of positive results point to the influence of the environment in which the persons live and refer to the influence of their profession, age and sex.

It was the aim of the present work to determine statistically the relation between the positive results and the environment, age and sex. It was also aimed to determine the influence of toxoplasmosis on the course of pregnancy.

Material and methods

Human sera were collected from pregnant women, women lying in childbed, blood donors and persons remaining in continuous contact with animal reservoirs (workers in meat plants) and examined. The examinations were conducted by the use of the complement fixation test according to Wassermann's technic and subsequently according to Kolmer's technic (cold method). The titre of the serum from 1 : 4 up was accepted as a positive result.

The statistical evaluation of relations between the examined characteristics (age, sex, environment) was based on Lancaster's method (1951) — on the distribution of χ^2 for multinominal table, more than binominal — onto χ^2 constituents. For the binomial tables was applied the method of statistical analysis based on the independence test of two — variable characteristics. The variance ration of the observed values (f) to the expected values (F) is
$$= \frac{(f - F)^2}{F}$$
 with the respective degree of freedom.

The value of P was given and it read from the distribution table of χ^2 (Pearson, Hartley, 1954) denotes the probability of obtaining by chance of a result not smaller than the observed result. In the present paper it was assumed to accept as essential those results of which $P < 0,95$ that means that our conclusions are based on a 5 per cent risk of error.

Results of examinations and discussion

A total number of 1307 persons from the Lublin Palatinate was examined. The following groups were separated:

- I. 375 persons — pregnant women in the VII, VIII or IX month of pregnancy — without a past gynecological history.

- II. 252 persons — women lying in childbed who delivered healthy children.
- III. 333 persons — in this group two subgroups were separated:
 - 1. Women lying in childbed who delivered prematurely or in time stillborns or children with developmental abnormalities.
 - 2. Women with gynecological case history (abortions, premature deliveries, stillborns and with developmental abnormalities).
- IV. 131 persons — blood donors — this group was accepted as a healthy population.
- V. 216 persons — workers from the Lublin meat plant — healthy human population remaining in continuous contact with breeding animals which may become a source of infection.

Jirovec, Jira, Fuchs et Peter, 1957 using the intradermal test examined women lying in childbed at the age from 16—50 years and obtained the following results: from 16—20 years 14% of positive results, 21—30 years — 32%, from 31—40 years — 42% and from 41—50 years — 33%. The percentage of positive results increased with years up to the age of 40 and henceforth decreased slowly.

Attempt was made to show the relation between the number of positive results and the age of the examined persons on the material studied by the authors. Table I illustrates the obtained results. The table shows that between the examined groups is a difference formed by the structure of the age ($\chi^2 = 151$; $P = 10^{-5}$).

The question arises, however, whether the number of positive results increases with age. In case of women lying in childbed and in pregnant women the percentage of positive results at the age of 20—30 amounts to 6.1, above the age of 30 years the percentage decreases to 1.5. The data referring to the age group above 30 years may not be reliable because the fact should be taken into consideration that the age above 30 years is not any more the period of the greatest fertility.

The highest percentage of invasions was found in the workers of meat plants at the age below 20 years — 3.2, at the age 20—30 years it amounted to 1.3 and at the age above 30 years it dropped to 0.92. This relation appears to be entirely different in the group of blood donors. The highest percentage of positive results is

observed in the group of persons above 30 years — 6.1, Table I shows the differences in percentages in the number of positive results depending on the age. Statistical analysis of the results presented in the table II does not confirm the increase of the number of positive results with the advancing years ($\chi^2 = 1.952$; $P = 0.74$). In none of the examined groups (women lying in child-bed, pregnant women, blood donors, workers of meat plants) neither in all those groups combined together was there found any influence of the age on the result of the complement fixation test.

Taking the results of the table I as a basis the separate groups were analyzed irrespectively of the age. The obtained results are shown in the table III.

In the total number of 1307 examined persons there were 6.5% of positive results, 3.1% of dubious results and 90.4% of negative results. The highest percentage of positive results 9.9%, contrary to conjectural estimates was found in the healthy population that is in blood donors. Starzyk et al., 1958 examined in Kraków blood donors using the dye test. A positive result was obtained in only 0.8% of cases. The results obtained by our examinations were compared with the results in the remaining groups and they were submitted to detailed statistical analysis. It appeared that the differences are not essential and that the obtained results are accidental ($\chi^2 = 3.30$; $P = 0.07$). The lowest percentage of positive results 2.6 was found in the group of pregnant women. Workers of meat plants and workers employed in cowsheds remain by their profession in continuous contact with breeding animals. Because the animals are in a high percentage infected with toxoplasmosis (Angeloff et al., 1957; Wysocka et Umiński, 1955; Seeman, 1959; Starzyk et al., 1957—1959;) it can be supposed that the persons who remain in contact with them are exposed to infection. In our studies concerned with the examination of the workers of meat plants the percentage of positive results was relatively low — 5.5. It should be stressed, however, that the majority of the examined persons worked in the meat industry not longer than one year.

Beverly and Beathie examined by the use of the dye test workers of slaughter houses and veterinary surgeons and they obtained 34% of positive results. However, Starzyk et al., 1957 examined 117 workers of the slaughter house in Kraków and

Table I

Dependence of result of complement fixation test on age of examined groups

Zależność wyniku odczynu wiązania dopełniacza od wieku badanych grup.

Result CFT Wynik O.W.D.	Age Wiek	Women lying in childbed and pregnant women Położnice i kobiety cię- żarne		Workers of Meat Plants Pracownicy Zakładów Mięśnych		Blood donors Dawcy krwi		Total num- ber liczba ogólna
		number examin. liczba zbad.	%	number examin. liczba zbad.	%	number examin. liczba zbad.	%	
positive dodatni	< 20	3	0.9	7	3.2	—	—	58
	20—30	24	6.1	3	1.3	5	3.8	
	> 30	6	1.5	2	0.9	8	6.1	
negative ujemny	< 20	45	11.4	70	32.4	—	—	657
	20—30	213	54.3	60	27.7	43	32.8	
	> 30	92	23.4	64	29.6	70	53.4	
dubious wątpliwy	< 20	1	0.2	4	1.8	—	—	24
	20—30	7	1.7	—	—	2	1.4	
	> 30	1	0.2	6	2.7	3	2.2	
Total Razem		392		216		131		739

Table II

Dependence of result of complement fixation test on age
Zależność wyniku odczynu wiązania dopełniacza od wieku

Result Wynik	Up to 20 years Do 20 lat	From 20 — 30 Od 20 — 30 lat	Above 30 years Powyżej 30 lat
positive dodatni	10	32	16
negative ujemny	115	316	226
dubious wątpliwy	5	9	10

Table III
Statistical presentation of results of complement fixation test
Statystyczne ujęcie wyników odczynu wiązania dopełniacza

Result CFT Wynik OWD	Pregnant in VII, VIII, and IX month of pregnancy		Women lying in childbed		Women with past gynecological history		Blood donors		Workers of Meat Plants	
	number exam. liczba zbad.	%	number exam. liczba zbad.	%	number exam. liczba zbad.	%	number exam. liczba zbad.	%	number exam. liczba zbad.	%
dodatni positive	f	2.6	19	7.5	31	9.3	13	9.9	12	5.5
	$\frac{(f - F)^2}{F}$	8.48		0.41		4.00		2.36		0.39
ujemny negative	f	94.6	225	89.2	294	88.0	113	86.2	194	89.9
	$\frac{(f - F)^2}{F}$	0.77		0.03		0.15		0.23		0.007
wątpliwy dubious	f	2.6	8	3.1	8	2.4	5	3.8	10	4.6
	$\frac{(f - F)^2}{F}$	0.08		0.001		0.57		0.19		1.54
Razem Total		375	252		333		131		216	

obtained only 2.55% of positive results using the same test. A high percentage of positive results (9.3%) was found in the group of women with a past gynecological history (undergone abortions, premature parturitions, deliveries of dead fetuses) and in women in whom the last parturition terminated before the time. This was confirmed by statistical analysis ($\chi^2 = 5.75$; $P = 0.02$). These results are in agreement with reports in the literature referring to the role of toxoplasmosis in gynecology. It was stated that acquired toxoplasmosis may lead to abortions, premature parturitions, death of fetuses or their developmental abnormalities (Finke, Schultze, Heitmüller, Westphal, Cecha).

It should be added, however, that not all support this opinion. Holmdahl conducted analogous studies but he sees no relation between abortions, premature parturitions and toxoplasmosis. However, on the basis of reports found in the literature and on the basis of the authors' own studies it seems that the role of toxoplasmosis in gynecology is unquestionable.

Table IV

Dependence of result of complement fixation test on environment
Zależność wyniku odczynu wiązania dopełniacza od środowiska

Environment Środowisko	Total number examined Ogólna liczba zbadanych	Result CFT Wynik OWD						
			+	%	—	%	±	%
village wieś	450	f	38	8.4	400	88.8	12	2.6
		$\frac{(f-F^{12})}{F}$	2.61		0.1		0.31	
city miasto	857	f	47	5.4	781	91.1	29	3.3
		$\frac{(f-F)^2}{F}$	1.54		0.05		0.09	
	1307	Total Razem	85		1181		41	

A greater number of infections with toxoplasmosis is recorded in rural areas, where the population remains in continuous contact with the animal reservoir. Piekarski, 1954 (Western Germany)

found that the percentage of infections is in the rural environment by 11.4 higher than in the urban environment (rural environment 22.2‰; urban environment 10.8‰). The results obtained by us are shown in the table IV. According to our studies the difference in the percentage of infections between a city and village is 3‰ (village 8.4‰; city 5.4‰). This difference is essential as shown by statistical analysis.

According to various authors, women become more commonly infected than men. In the studies conducted by Kozar, 1953 on the terrain of Gdańsk, the differences appear as follows: among the 336 examined men a positive result was recorded in 10.1‰ of the cases but in 345 women in 39.4‰ of the cases. Thalhhammer, 1957 conducting such studies in Vienna found 48.4‰ of positive results in women and 41.2‰ in men. Biagi in Mexico confirms the results. In 1952 he obtained 38.6‰ of positive results in women and 36.6‰ in men. All the mentioned results were obtained by using the intradermal test. The results obtained by the authors of the present paper are as follows: Positive result was recorded in 8.5‰ of women and in 6.1‰ of men; dubious in 3.2‰ of women and 2.8‰ of men. The differences are minimal what was confirmed by statistical analysis ($\chi^2 = 1.5$; $P = 0.22$).

Conclusions

1. In the examined material the percentage of positive results was in the complement fixation test 6.5, of dubious results 3.1, negative results — 90.4.
2. It was found that a high percentage (9.3‰) occurred in women with a past gynecological history and in women in whom the last parturition terminated prematurely. This was corroborated by statistical analysis ($\chi^2 = 5.75$; $P = 0.02$).
3. Higher percentage of infections with toxoplasmosis was recorded in rural areas (village — 8.4‰; city — 5.4‰). The difference confirmed by statistical analysis is essential ($\chi^2 = 4.2$; $P = 0.04$).
4. No increase of the number of positive results with the advance of years was found ($\chi^2 = 1.952$; $P = 0.74$).
5. It seems that the number of positive results is not dependent on sex ($\chi^2 = 1.5$; $P = 0.22$). Only percentage differences in the number of positive results were obtained (women 8.5‰, men 6.1‰).

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STRESZCZENIE

Celem pracy było określenie metodą statystyczną zależności między wynikami dodatnimi a środowiskiem, wiekiem i płcią oraz określenie wpływu toksoplazmozy na przebieg ciąży. Badania przeprowadzono przy pomocy odczynu wiązania dopełniacza. Przebadano 1307 surowic z woj. lubelskiego pochodzących od kobiet ciężarnych, położnic, dawców krwi oraz osób pozostających w ciągłym kontakcie z rezerwuarem zwierzęcym (pracownicy zakładów mięsnych). Uzyskane wyniki można streścić w następujących punktach:

1. Procent wyników dodatnich na ogólną liczbę przebadanych wynosi 6,5, wyników wątpliwych 3,1, wyników ujemnych 90,4. Największy odsetek wyników dodatnich stwierdzono u dawców krwi — 9,9% oraz w grupie kobiet z obciążoną przeszłością położniczą i u kobiet, u których ostatni poród zakończył się przedwcześnie (9,3%). U pracowników zakładów mięsnych odsetek wyników dodatnich wynosił 5,5%. Najmniejszy odsetek stwierdzono u kobiet ciężarnych bez obciążonej przeszłości położniczej — 2,6%.

2. Większy odsetek zakażeń toksoplazmozą zaobserwowano w środowisku wiejskim (wieś — 8,4%; miasto — 5,4%). Potwierdza to analiza statystyczna ($\chi^2 = 4,2$; $P = 0,04$).

3. Nie stwierdzono wzrastania ilości wyników dodatnich wraz z wiekiem ($\chi^2 = 1,952$; $P = 0,74$).

4. Stwierdzono minimalne różnice w ilości uzyskanych wyników dodatnich w zależności od płci (kobiety — 8,5%, mężczyźni — 6,1%). Posługując się analizą statystyczną stwierdzono, że różnica ta nie jest istotna ($\chi^2 = 1,5$; $P = 0,22$).

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